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Original article

Effects of tillage on microbial populations associated to soil aggregation in dryland spring wheat system

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ABSTRACT

Tillage may influence the microbial populations involved in soil aggregation. We evaluated the effects of no till (NT) and conventional tillage (CT, tillage depth about 7 cm) continuous spring wheat system on culturable heterotrophic bacterial communities predominant in microaggregates (0.25–0.05 mm) and on soil-aggregating basidiomycete fungi in aggregate-size classes (4.75–2.00, 2.00–0.25, and 0.25–0.05 mm) at 0–20 cm depth of a Williams loam (fine-loamy, mixed, Typic Argiustolls) in dryland Montana, USA. Enzyme-linked immunosorbent assay used to quantify antigenic response to basidiomycete cell walls, was higher in NT than in CT in 4.75–2.00 mm size class in 2007 and higher in all classes and years at 0–5 cm depth, but was not different between tillage, years, and classes at 5–20 cm. The culturable bacteria from microaggregates were subjected to a soil sedimentation assay to determine their soil binding capability. The proportion of isolates which can function as soil aggregators was higher in NT than in CT at 0–5 cm but was not different at 5–20 cm. Our results provide a first insight into the beneficial effects of dryland NT compared to CT in reducing soil disturbance and residue incorporation and enriching the proportion of microorganisms responsible for aggregation, especially at the soil surface.

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1. Introduction

Planting in the no till (NT) system is characterized by sowing of crops directly in the soil without tillage as opposed to conventional tillage (CT) where planting is done in tilled soil with most of the plant residues incorporated into the soil. Interaction between soil aggregation and carbon dynamics in different tillage managements has been intensively investigated; however, little is known about the composition and proportion of microorganisms in aggregates. Soil disturbance from tillage causes rapid disruption of macroaggregates (>0.25 mm size class) resulting in less accumulation of crop-derived C in free microaggregates (0.25–0.02 mm size class) that are formed within macroaggregates [51]. In NT, reduced soil disturbance and accumulation of crop residue at the soil surface result in improved soil structure and aggregation compared with CT. The slower macroaggregate turnover leads to more C sequestration in NT because of higher C concentration in macroaggregates than in microaggregates since macroaggregates are composed of microaggregates and labile organic matter, such as roots, fungal hyphae, and young plant residues [53]. The encapsulation of fine particulate organic matter by mineral particles and microbial products confers stability to the microaggregates under NT which can be bound together by transient, labile organic matter to form new macroaggregates [24].

Bacteria can survive in aqueous solutions within soil aggregate pores where they get energy and nutrients from encapsulated plant residues [60]. It has been reported that bacteria can bind soil particles and maintain soil aggregate stability by producing *in situ* extracellular polymeric substances (EPS) which are produced as capsular material and peripheral slime with adhesive properties [2,17]. The microbial basis of soil aggregation is likely similar to the process described by Mueller [39]: (1) microbiological degradation or alteration of organic materials, (2) attachment of cells to soil particles, (3) production of EPS, and (4) concerted construction of biofilms by microbial communities, resulting in aggregation of soil particles. The greater structural stability of the microaggregates than macroaggregates has been partly attributed to the presence of bacterial cells embedded in capsules of EPS often surrounded by a layer of clay particles [20]. Numerous studies have been focused on the effects of tillage on microbial biomass [22], microbial enzymatic activity [28], microbial diversity and community structure [34,42], but little information exists on culturable bacterial species that are predominant in the microaggregates or the proportion of isolates that can act as soil aggregators in relation to tillage system.

Several studies have reported the effects of tillage on arbuscular mycorrhizal fungi [35] or non-mycorrhyzal fungi [37] but little is

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known about tillage effects on saprotrophic basidiomycete fungi responsible for soil aggregation and nutrient cycling. Many saprotrophic basidiomycete fungi inhabiting fragmentary soil residues are important in decomposition and nutrient-release processes [6,43] because they secrete extracellular ligninolytic enzymes (laccases, manganese peroxidases, and cellulases) that attack the principal plant polymers lignin, cellulose or hemicellulose [59]. Electron micrographs show fungal hyphae can form extensive networks in soil and are often covered with extracellular polysaccharides which hold microaggregates together [21]. Besides their function in nutrient cycling, saprotrophic basidiomycetes can bind soil particles into aggregates through physical enmeshment and production of EPS [10] with "adhesive" properties [13]. Basidiomycete fungi have been demonstrated to play a role in aggregation of clay particles [19] and stabilization of soils [2,8]. Polyclonal antibodies raised against cell walls of a russuloid basidiomycete fungus isolated in agricultural cropland in eastern Montana, USA, were demonstrated to cross react significantly with many other russuloid species that were efficient soil stabilizers [9]. They were used to develop an enzyme-linked immunosorbent assay (ELISA) which can sensitively detect minute amount of antigens derived from the cell walls of these specific soil aggregating basidiomycete fungi in cropland soil, as low as 200 ng g^{-1} soil [9]. In this study, we report information on the survival and growth of saprotrophic basidiomycete fungi in soil aggregates under dryland spring wheat as influenced by tillage in the northern Great Plains.

We hypothesized that at the beginning of the growing season, the abundance of microbial populations functioning as soil aggregators would be greater in dryland continuous spring wheat after 3 years under NT compared to CT. Our objectives were to (1) evaluate the effects of tillage at 0–5 and 5–20 cm soil depths on soil aggregation and stability, (2) quantify the amount of soil aggregating basidiomycete fungi from different aggregate-size classes (4.75–2.00, 2.00–0.25, and 0.25–0.05 mm) using enzyme-linked immunosorbent assay (ELISA); (3) isolate the predominant heterotrophic bacteria in microaggregates (0.25–0.05 mm) by cultivation and use fatty acid methyl ester (FAME) profiling for their taxonomic identification, (4) procure adequate biomass of these isolates by cultivation and use soil sedimentation and photometry to determine their ability to aggregate soil, and (5) use DNA sequencing to confirm the identification of the soil aggregating culturable bacterial species.

2. Materials and methods

2.1. Site description, treatments, and soil sampling

The experimental site was located about 8 km northwest of Sidney, Montana, USA. Soil at the location was mapped as a Williams loam (fine-loamy, mixed, superactive, frigid Typic Argiustolls) [18]. Mean annual precipitation at the site is 320 mm, with about 80% occurring from April through September. Prior to initiation of this experiment, the site had been in a cereal grain-summer fallow rotation under fall and spring tillage for several decades.

This report provides results from a tillage treatment from a larger study investigating the interactions of tillage and cultural management systems on four crop rotations. The experiment was initiated in 2004 in a randomized complete block design with three replications. The whole plot treatment was tillage system, conventional tillage and no tillage. Split-plot treatments were four crop rotations with two cultural management systems. For this study, only the effect of tillage on microorganism communities and population under spring wheat with conventional cultural management was considered. Individual split-plot size was 12 m by 12 m. Nitrogen fertilizer was broadcast to spring wheat at 78 kg N ha^{-1} as urea in mid-April each year, prior to tillage. Preplant conventional tillage was conducted in tilled plots with a field cultivator equipped with C-shanks hooked to a 45 cm wide sweeps and coil-tooth spring harrows with 60 cm bars. Conventional tillage depth of about 7 cm was controlled by stabilizer wheels on the field cultivator frame. Spring wheat 'Reeder' was planted at 78 kg seeds ha^{-1} in mid-late April with a 3.1 m wide drill in a row spacing of 20.3 cm. The drill was equipped with double-shoot Barton (http://www.flexicoil.com/barton.asp) disk openers for low soil disturbance, single-pass seeding and fertilization. Phosphorus fertilizer as monoammonium phosphate (11% N and 52% P) was applied at 56 kg P ha^{-1} and K fertilizer as muriate of potash (60% K) was applied at 48 kg K ha^{-1} to spring wheat at planting in a band about 5 cm below and to the side of the seed row. Spring wheat residue was evenly spread with a combine equipped with a chopper-spreader at harvest each year.

Soil samples were collected with a hand probe (5 cm inside diameter) from 5 places to a depth of 20 cm in the central rows of each plot after removing surface residues in early spring of 2005, 2006 and 2007, separated into 0–5 cm and 5–20 cm depths, and composited within a depth. Samples were processed within 24–48 h for determinations of soil aggregation and microbial communities. Aggregates were separated by dry sieving of moist soil after drying at 4 °C [36] and the aggregate proportion (g aggregate kg^{-1} soil) was measured in 4.75–2.00, 2.00–0.25, and 0.25–0.05 mm aggregate-size classes. Mean weight diameter (MWD), used as an index of aggregate stability, was calculated according to the procedure described by Kemper and Rosenau [29].

2.2. Isolation of bacteria

Culturable bacteria occurring at the highest population levels in the microaggregates were isolated using spiral plating technique according to [11] with slight modifications. Briefly, microaggregates (1 g) were agitated in $MgSO_4$ buffer (0.1 M, pH 7.3) with glass beads (0.5 mm) for 6 h at 4 °C on a shaker at 200 rpm to release the bacteria. Soil suspensions were diluted by a factor of 100 with buffer and spiral plated on low nutrient R2A medium (Difco Laboratories, Sparks, MD, USA) [44]. R2A medium was previously used to grow a wider spectrum of bacteria to grow without suppressing the slow-growing species, thereby achieving a higher recovery of bacteria [34], and was also used to isolate surfactant producing bacteria [40]. The basic concept of spiral plating was to continuously deposit a known volume of sample on a rotary agar plate in the form of an Archimedes spiral [23,26]. The amount of sample decreased evenly while the dispensing stylus was moved from the center to the edge of the rotating agar plate. From the beginning to the end of the spiral at the periphery of the plate represented a 3 logarithmic unit dilution [26] when 150 μl aliquots of soil suspensions were plated, thus 10^5 cells g^{-1} soil represented a typical population level at which the colonies which were considered the predominant isolates were obtained. For each year and each soil depth (0–5 cm or 5–20 cm), a total of 66 colony-forming units growing at the end of the spiral at the periphery of the rotary agar plates were collected for each treatment (NT and CT) after 48–72 h of incubation at 28 °C. A total of 792 isolates were cultured and purified for the two tillage treatments at the two soil depths (0–5 and 5–20 cm) in 2005, 2006 and 2007. Among the 792 isolates, only 747 were identified by (FAME) profiling (see 2.4) and were assayed in soil sedimentation to determine their potential to aggregate soil. Only the proportion of isolates which showed soil aggregating ability was compared between the two tillage treatments at the two different soil depths. All isolates were stored at −80 °C in Luria–Bertani medium amended with 15% glycerol.

2.3. Sedimentation assay

A sedimentation assay was used under controlled laboratory conditions to screen the culturable bacterial isolates that showed the ability to aggregate soil. Pure cultures of isolates (100 mg) grown for 24–48 h on 1% TSBA (trypsin soy broth agar, Difco Laboratories, Sparks, MD, USA) were washed once with deionized water [32] in order to eliminate residual nutrients from the culture medium. Cells were counted using a hemacytometer before adding to glass tubes (20 × 150 mm, Corning, NY) containing 10 ml of deionized water and 1.25 g of sieved soil (<0.05 mm size-class) to obtain final cell concentrations of 10^6 cells ml^{-1}. The cell concentration of 10^6 cells ml^{-1} was used to amend soil for assay the minimum cell concentration at which sedimentation of soil particles could occur, as established in preliminary studies with a range of bacteria species. Soil collected along a stream bank at Sidney, Montana (14% clay, 14% silt, and 72% sand) was used for the aggregation assay with the bacteria because of its low shrink/swell capacity [3]. Triplicate samples were prepared for each individual identified isolate. A control without bacteria was prepared in a similar manner for each individual isolate.

Tubes were vortexed for 10 s at 2250 rpm (Vortex Genie 2, Scientific Industries, USA) and the mixture was allowed to settle for 5 min at room temperature. Images of the reflected light (Universal/Hi-vision fluorescent light F32T8/TL735, Philips, NY, USA, light intensity 7.435 ± 0.064 Rad [watt/m^2]) for multiple samples were captured using a digital camera (Nikon, model D-80, Japan) with night vision settings (near infrared, 800–1000 nm) at 24.78°C ± 0.746. The captured images were calibrated by referring white (255 in gray-scale value) and black (0 in gray-scale value) image spots. Adobe Photoshop (version 7.0) was used for the conversion of the images in a gray-scale value of each target solution into the reflectance (expressed in %) that directly correlates to relative differences of the solution density; 100% reflectance for maximum density and 0% for minimum density. A reflectance ratio (% reflectance of the solution with bacterial cells added divided by the reflectance of control solution without cells added) was calculated for each isolate and values were averaged for all the isolates for each species. The solution with reflectance ratio >1.5 was established as a threshold which represents species that aggregate soil. Ratio below 1.5 represented the activities of species that were inconsistent or marginal in aggregating ability. To assure that identification made by FAME profiling corroborates with molecular-based methods of identification, the most efficient soil aggregating species identified by FAME [species with a reflectance ratio >1.5 and highest similarity index (SIM)] were further processed for DNA sequencing to confirm their identity and position in a phylogenetic tree having strain types from the Ribosomal Database Project [16].

2.4. Identification by FAME and DNA analyses

All predominant culturable bacterial isolates from microaggregates were identified using fatty acid methyl ester (FAME) profiling [7]. FAMEs were obtained by saponification, methylation and extraction following the MIDI system (Microbial Identification System, Inc. Newark, NJ, USA). MIDI Microbial Identification Software (Sherlock Aerobic Bacterial TSBA50 Library; Microbial ID Inc.) was used for the identification of the isolates. *Bacillus maroccanus* (ATCC # 25099) and *Stenotrophomonas maltophilia* (ATCC # 13637) were used as references. Only scores of SIM ≥ 0.500 were considered a good match [41]. Isolates having SIM < 0.500 or that were unidentifiable by FAME due to lack of information in MIDI Aerobic Bacterial Library TSBA50 were not investigated in this study.

To corroborate identifications made by MIDI, molecular-based methods of identification were additionally used. DNA from the isolates was extracted using a Qiagen (Valencia, CA, USA) DNeasy Tissue kit. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene region used primers16S-27f (5′-GAGTTTGATCCTGGCTCAG-3′) [31] and 16S-960r (5′-GCTTGTGCGGGYCCCCG-3′) [45] with the following cycling conditions: 95 °C (10 min); 25 cycles of 94 °C (30 s), 56 °C (30 s), 72 °C (2 min); and then 72 °C (2 min). A 50 μL reaction was performed for each isolate, and PCR products were purified using a QIAquick PCR Purification kit (Qiagen, Valencia, CA, USA). Purified templates were sequenced in two directions with an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA), using the same primers listed above. Isolate DNA sequences generated in this study are available from the corresponding author, and were aligned using CLUSTALW [58]. Maximum Parsimony (MP) analysis of the data set was performed using PAUP* v. 4.0b8 [57]. The heuristic MP search employed 500 random taxon addition sequences and the tree-bisection-reconnection (TBR) branch-swapping algorithm. All characters were weighted equally and insertion/deletion events, regardless of their length, were treated as one mutational event [50]. A 10,000 replicate "fast" stepwise-addition bootstrap analysis was conducted to assess clade support.

2.5. ELISA

The ELISA used for the detection and quantification of the amount of specific soil aggregating basidiomycete fungi in soil has been described previously in details according to Caesar-TonThat et al. [9]. Soil samples (12.5 mg) were prepared in 1 mL of carbonate buffer (20 mM $NaHCO_3$, 28 mM Na_2CO_3, pH 9.6). Absorbance was read at dual wavelengths of 450/655 nm with a microplate reader (Bio-Rad, Hercules, CA, USA). The amount of fungi was expressed in μg g^{-1} soil as determined from a standard curve generated from known amounts of antigens from basidiomycete cell walls. The aggregate samples were analyzed at least 3 times.

2.6. Statistical analysis

The Honestly Significant Difference test of Tukey–Kramer in ANOVA procedure was used to analyze data on soil aggregate distribution, soil sedimentation assay, and fungal quantification at 0–5 and 5–20 cm depths, with significance level evaluated at $P \leq 0.05$ using the JMP statistical software package (version 6.0, 2005, SAS Institute Inc., Cary, NC, USA). Tillage was considered as the main plot, aggregate-size class as the split-plot, and year as the repeated measure treatment for the analysis. The PROC GLIMMIX (SAS Institute Inc., Cary, NC, USA) statistical procedure that utilizes a logit model [33] was used to fit the proportion of the predominant aggregating soil bacterial isolates under the 2 tillage managements (CT and NT) over 3 years (2005–2007) and 2 soil depths (0–5 and 5–20 cm). Tillage management and cropping year were analyzed as factors affecting the proportion of culturable bacterial isolates that can function as soil aggregators using a generalized linear model. To calculate the species diversity identified by FAME profiles, the Shannon and Weaver [48] index $H = -\sum (ni/N)\ (\log ni/N)$ was used, in which ni is the number of individuals observed for each species and N is the total number of individuals observed in each treatment. Rarefaction curves were constructed to compare the richness of the soil aggregating culturable species of the 2 tillage managements (CT and NT) over 3 years (2005–2007) and 2 soil depths (0–5 and 5–20 cm). Rarefaction calculations were done using the software Species Diversity and Richness III, version 3.03 [55]. The program uses the rarefaction equations described by Heck et al. [25].

3. Results

The distribution of soil among the different size classes of aggregates showed that at 0–5 cm soil depth, aggregate proportion in the 4.75–2.00 mm size class was in general greater in NT than in CT in all 3 years but was only significantly greater in the third year of the study (2007), with a subsequent decrease in the 2.00–0.25 mm size class (Table 1). The mean-weight diameter (MWD) was in general greater in NT than in CT in all 3 years, but was only significantly greater in 2007. Overall, aggregate proportion was 1.6 times greater in NT (333.74 g kg^{-1}) than CT (209.00 g kg^{-1}) and greater in 2005 and 2007 than in 2006 in the 4.75–2.00 mm size class. Similarly, MWD was overall greater in NT (1.86 mm) than in CT (1.57 mm) and greater in 2005 and 2007 than in 2006. At 5–20 cm depth, aggregate proportion and MWD were not influenced by tillage (Table 2). Both aggregate proportion in the 4.75–2.00 mm size class and MWD were greater in 2007 than 2005 and 2006.

Table 3 indicates the amount of soil aggregating basidiomycete fungi detected by ELISA in aggregate-size classes of 4.75–2.00, 2.00–0.25, and 0.25–0.05 mm from soil collected under CT and NT in spring 2005, 2006, and 2007 at 0–5 and 5–20 cm depth. At 0–5 cm soil depth, the amount of fungi was greater in NT than in CT in 2006 and 2007 in the 4.75–2.00 mm class; for example, there was 1.3 times increase in 2006 (254.65 μg g^{-1} under NT versus 192.60 μg g^{-1} under CT) and a1.6 times increase in 2007 (290.22 μg g^{-1} under NT versus 186.73 μg g^{-1} under CT). In 2007, the amount of fungi was greater under NT than CT in the 2.00–0.25 mm class (331.30 μg g^{-1} under NT versus 201.76 μg g^{-1} under CT) and in the 0.25–0.05 mm class (236.32 μg g^{-1} under NT versus 190.16 μg g^{-1} under CT). Overall, fungi amount was greater in NT than in CT and greater in 2006 than in 2005 in 2.00–0.25 and 0.25–0.05 mm size classes. At 5–20 cm depth, basidiomycete amount was not different between tillage, aggregate-size classes, and years.

The *in vitro* soil sedimentation assay has allowed the culturable bacterial isolates (747) to be characterized for their capability to aggregate soil. Results show evidence that tillage and year significantly influenced the proportion of the predominant culturable soil aggregating bacteria in microaggregates and that the interactions tillage × soil depth and year × soil depth were significant (Table 4). Tests for the interaction of tillage management and soil depth indicate a tillage effect at 0–5 cm soil depth but not at 5–20 cm (Fig. 1); at 0–5 cm soil depth, the estimated proportions of the culturable soil aggregating bacteria were 0.09 under CT and 0.29 under NT. Tests for the interaction of year and soil depth indicate a year effect at 5–20 cm and not at 0–5 cm depth (Fig. 2); at 5–20 cm, the proportions of the culturable soil aggregating bacteria were 0.07 in 2005 and 0.31 and 0.27 in 2006 and 2007 respectively.

Among the 747 predominant culturable bacterial isolates from the two tillage treatments (CT and NT) at two soil depths (0–5 and 5–20 cm) in 2005, 2006 and 2007 which were identified to species by FAME profiling, only 160 isolates were selected because they demonstrated efficiency to aggregate soil particles by the soil sedimentation assay. Table 5 indicates the distribution of these culturable soil aggregating isolates (species abundance) into 12 different gram-negative species (α, β, and γ Proteobacteria, and Flavobacteria) and 14 gram-positive species (Bacilli including family Bacillaceae, Brevibacillaceae, Planococcaceae, Paenibacillaceae, and family Microbacteriaceae of Actinobacteria). At 0–5 cm depth, the number of soil aggregating culturable species found in microaggregates under NT was 2.2 times higher than in CT and at 5–20 cm depth, 1.3 times more species were found in CT than in NT. At 0–5 cm soil depth, species diversity indices of NT 0–5 cm was greater than CT 0–5 at $P = 0.089$, but at 5–20 cm depth, there was no difference between the two tillage treatments. In the comparison between soil depths, diversity index of NT 0–5 cm was greater than NT 5–20 cm at $P \leq 0.05$ and there was no significant difference between CT 0–5 cm and CT 5–20 cm. Fig. 4 indicates the rarefaction curves created for the treatments NT 0–5 cm, CT 0–5 cm, NT 5–20 cm, and CT 5–20 cm. Rarefaction curves indicate that for all treatments, a plateau was approached and that species accumulations are nearly an asymptote (zero or low slope). Comparison of the treatments shows that the species number was the highest in NT 0–5 cm and the lowest in CT 0–5 cm.

To corroborate the identification by FAME of the soil aggregating species (Table 5), the 16S rRNA amplification region of all the species were DNA sequenced and analyzed. Fig. 3 indicates the position of these isolates in the maximum parsimony tree showing the relationship to reference gram-negative and gram-positive bacteria. In general, DNA analysis of the isolate sequences matched with the FAME analysis. Among the discrepancies found between the two approaches of identification, DNA analysis placed the members of families Paenibacillaceae (isolate 06ZCL1B7 and 07TCL1B3) and Brevibacillaceae (06ZCL2C12 and 06ZCL1B6) in the Bacillaceae family cluster.

Table 1
Effects of tillage on soil aggregate proportion and mean-weight diameter of aggregates at the 0–5 cm depth.

Year	Tillage[a]	aggregate proportion in size class (g Kg^{-1} soil)			Mean-weight diameter (mm)
		4.75–2.00 mm	2.00–0.25 mm	0.25–0.05 mm	
2005	CT	319.43ab[b]	658.27cd	20.83ab	1.82bc
	NT	411.77a	569.77d	16.17ab	2.04ab
2006	CT	68.73d	881.10a	48.67a	1.23e
	NT	159.13cd	821.63ab	17.83ab	1.46de
2007	CT	238.83bc	738.50bc	20.83ab	1.64cd
	NT	430.33a	552.83d	14.50b	2.08a
Means					
2005		365.60a	614.02b	17.77a	1.93a
2006		113.93b	851.37a	33.25a	1.35b
2007		334.58a	645.67b	17.67a	1.93a
	CT	209.00b	759.09a	29.62a	1.57b
	NT	333.74a	648.08a	16.17a	1.86a

Significant Difference procedure of Tukey and Kramer at $P \leq 0.05$.
[a] Tillage: CT, conventional till; NT, no tillage.
[b] Within a set in a columm, numbers followed by different letters are significantly different by the Honesty.

Table 2
Effects of tillage on soil aggregate proportion and mean-weight diameter of aggregates at the 5–20 cm depth.

Year	Tillage[a]	Aggregate proportion in size class (g Kg^{-1} soil)			Mean-weight diameter (mm)
		4.75–2.00 mm	2.00–0.25 mm	0.25–0.05 mm	
2005	CT	335.33ab[b]	631.67ab	20.33a	1.85ab
	NT	317.67ab	661.67ab	19.00a	1.80ab
2006	CT	182.57b	805.33a	8.70b	1.60b
	NT	211.13b	782.03a	6.80b	1.53b
2007	CT	442.47a	554.87b	0.80b	2.12a
	NT	440.43a	557.03b	0.80b	2.11a
Means					
2005		326.50b	646.67b	19.67a	1.83b
2006		196.85c	793.68a	7.75b	1.56c
2007		441.45a	555.95b	0.80b	2.12a
	CT	320.12a	663.96a	9.94a	1.83a
	NT	323.08a	666.91a	8.87a	1.84a

Significant Difference procedure of Tukey and Kramer at $P \leq 0.05$.
[a] Tillage: CT, conventional tillage; NT, no tillage.
[b] Within a set in a columm, numbers followed by different letters are significantly different by the Honesty.

Table 3
Effect of tillage on the amount of basidiomycete fungi in aggregate-size classes at 0–5 and 5–20 cm soil depth.

Year	Tillage[a]	Amount of basidiomycetes in aggregate size class (μg g^{-1} aggregates)					
		0–5 cm	5–20 cm	0–5 cm	5–20 cm	0–5 cm	5–20 cm
		4.75–2.00 mm		2.00–0.25 mm		0.25–0.05 mm	
2005	CT	197.47c[b]	169.26a	174.49c	151.80a	186.60b	149.23a
	NT	228.60bc	187.72a	211.56bc	172.41a	223.23ab	174.42a
2006	CT	192.60c	175.20a	222.15bc	181.65a	209.27ab	171.41a
	NT	254.65ab	200.40a	298.88ab	200.47a	247.06a	182.15a
2007	CT	186.73c	163.11a	201.76c	183.44a	190.16b	153.16a
	NT	290.22a	201.90a	331.30a	194.39a	236.32a	173.56a
Means							
2005		213.04a	178.50a	193.03b	162.11a	205.01b	161.82a
2006		223.62a	187.80a	260.52a	191.06a	228.17a	176.78a
2007		238.47a	182.50a	266.53a	188.91a	213.24ab	163.36a
	CT	192.27b	169.19a	199.47b	172.69a	195.41b	157.93a
	NT	257.82a	196.68a	280.58a	189.09a	235.54a	176.71a
Significance							
Year		*	*	*	*	*	*
Tillage		***	*	***	*	***	*
year × tillage		*	*	**	*	*	*

*Significance at $P \leq 0.05$; **significant at $P \leq 0.01$ and ***significant at $P \leq 0.001$.
[a] Tillage: CT, conventional tillage; NT, no till.
[b] Within a set in a column, numbers followed by different letters are significantly different by the Tukey and Kramer Honestry Significant Difference test.

4. Discussion

Our data indicate that large macroaggregate (4.75–2.00 mm class) formation and mean-weight diameter (MWD) (Table 1) were in general higher in NT than in CT in all three years and there was an increasing trend for the amount of basidiomycete fungi (Table 3) and proportion of aggregating predominant culturable bacteria (Fig. 2) in aggregates under NT than CT at 0–5 cm soil depth. This suggests that NT spring wheat system in dryland eastern Montana, USA, improves soil aggregation and aggregate stability and increases the amount/proportion of soil aggregating microorganisms in aggregates at the soil surface. In similar dryland conditions in the Northern Great Plains, Sainju et al. [47] investigated tillage effects on dryland spring wheat residue and soil carbon fractions and proposed that lower precipitation, shorter growing season and slower decomposition of residue in the soil could cause minimum effect of tillage on crop residue production and soil organic C (SOC) content in aggregate-size fractions, at 0–5 cm. However, they demonstrated that macroaggregate formation (4.75–2.00 mm class) and soil particulate organic carbon (POC) concentrations in macroaggregates are higher in NT than in CT at the 0–5 cm. Although SOC and POC concentrations were not measured in our study, we also expect that POC is higher in macroaggregates under NT than CT. At the subsurface layer (5–20 cm), there was no influence of tillage in soil aggregation and aggregate stability between NT and CT in all three years (Table 2) and tillage had no effect on the amount/proportion of the soil aggregating basidiomycete fungi and culturable bacteria, probably because of the limited tillage depth (about 7 cm) in CT that reduced fresh residue incorporation into the soil and deep soil disturbance.

The greater proportion of saprotrophic basidiomycete fungi in soil aggregates in NT than in CT at 0–5 cm depth suggests that NT cultivation induced a means of survival for soil basidiomycete fungi better than CT systems. An NT system with inputs of high lignin content wheat residues (225 g kg^{-1}) [30] would provide a good source of nutrients for these fungi that are known to produce ligninolytic degrading enzymes to break down lignified plant materials for their utilization. They are sensitive to soil disturbance due to tillage [9], as tillage decreases basidiomycete populations in response to increase in mitosporic species, such as *Penicillium* spp. and *Fusarium* spp., which can withstand soil disturbance by increasing the production of survival spores [56] and which also have much smaller mycelial individuals often occupying single organic particles or seeds in soil. They can also probably survive and grow better in NT than in CT in the semi-arid regions dryland cropping system in eastern Montana, since NT generally provides a more favorable environment for fungi to grow by increasing water

Table 4
Analysis of variance for the proportion of the predominant soil aggregating bacteria isolated from microaggregates.

Source	F value	Pr > F
Tillage[a]	28.20	<0.0001
Year[b]	5.07	0.02
Tillage × year	0.76	0.48
Soil depth[c]	1.03	0.32
Tillage × soil depth	15.51	0
Year × soil depth	5.83	0.01
Year × Tillage × soil depth	0.8	0.46

[a] Tillage: no till and conventional tillage.
[b] Cropping years: 2004–2007.
[c] Soil depth: 0–5 cm and 5–20 cm.

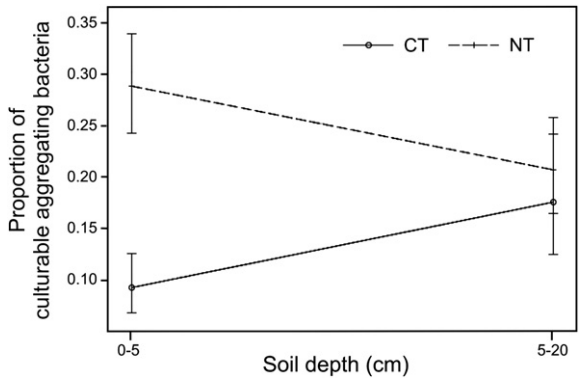

Fig. 1. Proportion of culturable soil aggregating bacteria as affected by tillage (no till, NT and conventional tillage, CT) and soil depth (0–5 and 5–20 cm) interaction.

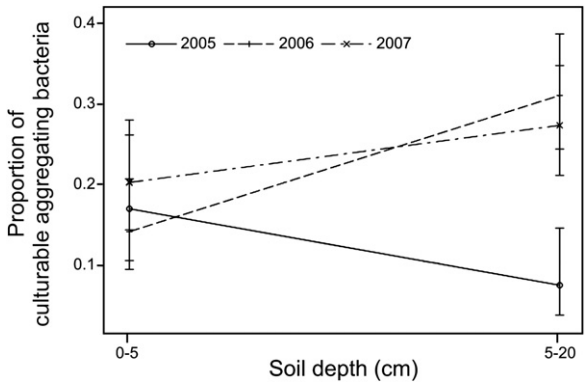

Fig. 2. Proportion of culturable soil aggregating bacteria as affected by year (2005–2007) and soil depth (0–5 and 5–20 cm) interaction.

content compared with CT in the dryland cropping system [27,46]. It has been reported that some basidiomycetes are adapted to desiccating environments by their ability to produce copious amount of mucilage allowing them to grow at moisture potentials as low as −4 to −8 MPa [61].

Saprotrophic basidiomycetes are well documented to control the mineral and energy cycling of plant litter and to function as regulators for the release of pulse nutrients to the soil, in both time (immobilization of nutrients into fungal biomass [14]) and space (translocation and redistribution of carbon and nutrients through hyphal assemblages called rhizomorphs or strands [12]). Under NT dryland spring wheat system where residue inputs are frequent (i.e. yearly) and the soil at 0–5 cm depth is not disturbed, the increase of basidiomycete populations in all aggregates size classes suggests that, during the course of residue decomposition, the fungi forms an extensive branching mycelial network [1] which can entangle soil particles to form aggregates where they probably reallocate and slowly redistribute nutrients immobilized within their tissues. These activities could provide more easily available carbon and minerals to bacteria inhabiting the microaggregates for their growth and may favor the growth and survival of specific bacterial populations involved in soil aggregation. Although no evidence was provided in this study to support the hypothesis that more carbon resources and minerals were transported to the microaggregates in NT treatment than CT by basidiomycete fungi, our data are in accordance with Six et al. [52] who demonstrated that there is greater accumulation of crop-derived C in free microaggregates in NT than in CT.

Under CT with crop residues incorporated every year into the soil, the contact area between soil and organic matter increases and wheat residues decompose at higher rates with a more rapid loss of nutrients [15] and mineral leachate [54]. Saprotrophic basidiomycete populations could suffer under CT because they rely on complex plant debris for a major part of their diet, contrary to shorter-lived and ephemeral molds that prefer simple carbohydrates from readily decomposed materials for consumption which are more

Table 5
Distribution of the predominant culturable soil aggregating bacterial isolates from microaggregates of soil cropped to continuous spring wheat from 2004 to 2007 under different tillage[a] management, at 0–5 and 5–20 cm soil depth.

Class	Identification by FAME	CT 0–5[a]	NT 0–5	CT 5–20	NT 5–20	Reflectance ratio[b]	Identification by DNA sequencing[c]
Gram negative							
α-Proteobacteria	*Rhizobium radiobacter*	0	1	0	0	2.065	5ZCL1a1-1 (0.766)
	Brevundimonas vesicularis	0	0	3	0	1.543	6TCL2b23 (0.466)
	Novosphingobium capsulatum	0	0	4	1	2.926	6ZCL2b20 (0.627)
	Sphingomonas sanguinis	0	0	0	2	2.001	6ZCL2c8 (0.699)
β-Proteobacteria	*Variovorax paradoxus*	0	2	0	0	1.657	7ZCL1a14 (0.510)
	Burkholderia cepecia	0	0	1	0	2.077	6TCL2b7 (0.671)
	Vogesella indigofera	0	0	1	0	1.579	5TCL1a3-3 (0.502)
γ-Proteobacteria	*Pseudomonas fluorescens biotype A*	0	2	0	0	1.599	6ZCL1a15 (0.629)
Flavobacteria	*Zobellia uliginosa*	0	1	0	0	1.548	7ZCL1c9 (0.404)
	Chryseobacterium balustinum	0	3	0	1	1.966	7ZCL1c22 (0.693)
	Chryseobacterium indoltheticum	2	7	1	2	1.991	7ZCL1c21 (0.682)
	Flavobacterium hydratis	0	1	0	0	1.974	5ZCL1a3-5 (0.379)
Gram positive							
Bacilli (Bacillaceae)	*Bacillus atrophaeus*	2	4	1	1	4.310	5TCL1a10 (0.890)
	Bacillus cereus	0	1	0	0	6.075	6ZCL1b10 (0.591)
	Bacillus flexus	0	2	3	6	1.799	6TCL2a3 (0.585)
	Bacillus niacini	1	2	0	1	2.280	NA
	Bacillus pumilus	2	7	4	6	1.943	7ZCL2b18 (0.710
Bacilli (Brevibacillaceae)	*Brevibacillus choshinensis*	3	9	16	18	2.592	6ZCL2c12 (0.867)
	Brevibacillus parabrevis	1	4	5	3	5.282	6ZCL1b6 (0.723)
Bacilli (Planococcaceae)	*Kurthia sibirica*	0	1	0	0	2.856	6ZCL1b18 (0.481)
Bacilli (Paenibacillaceae)	*Paenibacillus azotofixans*	0	0	1	0	1.909	7TCL2a15 (0.671)
	Paenibacillus macerans	1	0	0	0	4.337	7TCL1b3 (0.809)
	Paenibacillus pabuli	5	3	1	0	1.954	6TCL1b9 (0.833)
	Paenibacillus validus	0	2	4	3	4.620	6ZCL1b7 (0.752)
Actinobacteria (Microbacteriaceae)	*Microbacterium hominis*	0	1	0	0	1.953	5ZCL1a2-2 (0.723)
	Microbacterium lacticum	0	0	1	0	1.671	6TCL2a6 (0.926)
Diversity index[d]		1.92b*	2.60 a*	2.18 ab	1.90 b		

*$P = 0.089$, otherwise $P \leq 0.05$.

[a] Management: CT 0–5 and NT 0–5, conventional till and no till at 0–5 cm soil depth; CT 5–20 and NT 5–20, conventional till and no till at 5–20 cm soil depth.

[b] Average of reflectance ratio (reflectance measurement of soil suspension containing 106 cells/mL after 5 min of sedimentation time divided by reflectance measurement of soil suspension without bacteria added) obtained from all isolates of each soil aggregating species.

[c] Isolates from species that have the highest similarity index and the reflectance ratio >1.5 were DNA sequenced and analyzed. Relationship of unknown isolates and type specimens are shown in Fig. 1. The similarity index indicated between parentheses was based on MIDI Aerobic Bacterial Library TSBA50; NA, not available.

[d] Shannon–Wiener's diversity index.

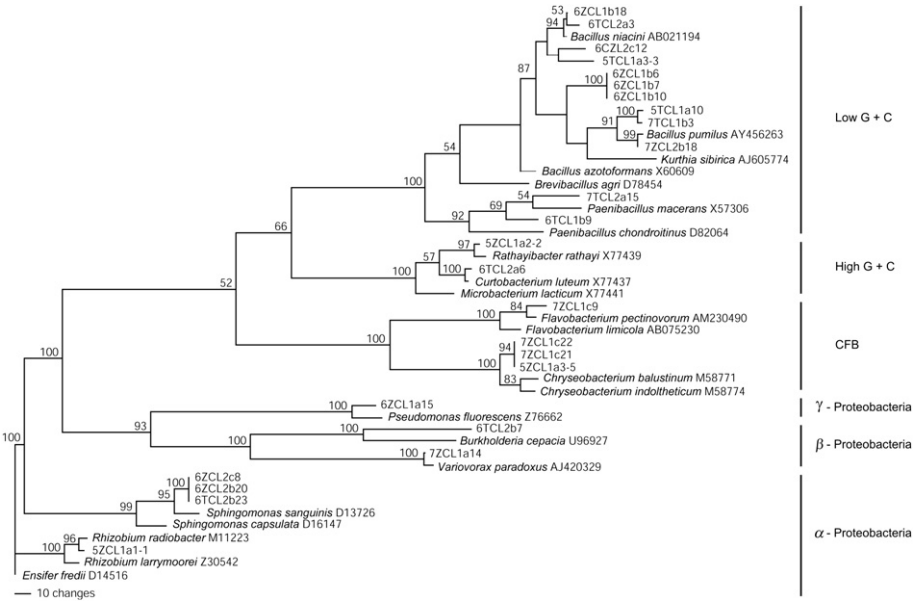

Fig. 3. Single most parsimonious tree, 2878 steps in length, resulting from the analysis of 16S rRNA gene sequences (838 aligned bases; 364 of these parsimony informative) from 12 soil-aggregating culturable bacteria and 17 known type strains of related bacteria. Isolates from this study are shown in bold, while taxa used as phylogenetic placeholders (shown in italics, with GenBank accession numbers following specific epithet) are from the Ribosomal Database Project II Hierarchy Browser collection of sequenced Type Strains [16]. Bootstrap values (>50%) are shown above branches. CFB, Cytophaga-Flavobacterium-Bacteroides group.

available under CT. The rapid decomposition of residues in CT in the surface soil layer could result in low storage of nutrients in microaggregates, thus affecting the growth and survival of bacterial communities that require sufficient energy resources to produce extracellular components acting as soil binding agents. A possible explanation for higher proportions of soil aggregating bacteria in CT 5–20 cm compared to CT 0–5 cm (0.17 versus 0.09) could be that below tillage depth (approximately 7 cm), the negative impact of CT treatment observed in the upper soil layer (0–5 cm soil depth) is alleviated by slow decomposition and the rhizosphere effect (rhizodeposits), which provide necessary nutrient resources for soil aggregating bacteria to proliferate. In addition, the non significant difference in the amount of basidiomycetes in aggregates of NT 5–20 cm and CT 5–20 cm (Table 3) was probably because these saprotrophic fungi are more frequently found in plant debris at or near the soil surface than in greater depth. However, saprotrophic fungi associated to water stable aggregates could be found colonizing dead roots of *Pinus radiata* D. Don. planted on sandy soils to a depth of several feet [5].

The relatively higher proportions of culturable soil aggregating bacteria in NT compared with CT at 0–5 cm depth in microaggregates (Fig. 1) suggests that NT increased the population of these specific bacteria at the surface soil probably by increasing particulate organic carbon (POC) content that constitutes the source of energy and nutrients for microorganisms [4,52]. As a result, specific bacterial species probably increased mucilage production responsible for increased aggregation under NT. Mendes et al. [36] reported that the greatest rates of enzyme activities tended to be found inside the microaggregates where the microorganisms are biologically active and significantly involved in processing soil C [27].

Rarefaction analysis created for each of the tillage treatments over 3 years at the 2 soil depths (Fig. 4) revealed that the culturable soil aggregating bacterial species analyzed in this study were sufficient to describe the bacterial diversity at the species level. The rarefaction curves showed indication of reaching nearly an asymptote, thus demonstrating that the number of culturable bacteria was sufficiently sampled and that no species can be expected if additional isolates were to be analyzed. The slope of CT 0–5 cm curve was shallower than those of the curves obtained for NT 0–5 cm, NT 5–20 cm and CT 5–20 cm, suggesting that a small number of species were dominating in the treatment CT 0–5 cm.

Many culturable bacteria identified by FAME and demonstrated to have high potential for soil aggregation from this study were also found in other studies. Sessitsch et al. [49] demonstrated the presence of *Rhizobium* and *Shingomonas* spp of the class of α Proteobacteria in soil particle fractions of various size using cultivation-independent techniques. Miller et al. [38] found *Pseudomonas* spp in soil aggregates from grassland and arable cropping systems. *Bacillus cereus*, *Bacillus pumilus*, *Brevibacillus choshinensis*, and species from genera *Pseudomonas* and *Chryseobacterium* were also found previously in soil aggregates in a previous study [11].

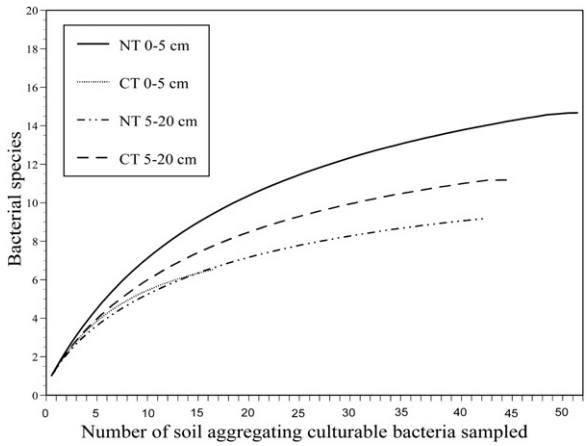

Fig. 4. Rarefaction curves constructed for the different tillages treatments (NT, no tillage and CT, conventional tillage) at different soil depths (0–5 cm and 5–20 cm) showing the expected number of species as a function of the number of soil aggregating culturable bacteria sampled.

In recent years, culture-independent methods used to study bacterial communities have received particular attention because the majority of soil bacteria, i.e., more than 99%, are not accessible by cultivation methods. However, molecular approaches are not yet fully feasible to establish the potential to form macroaggregates of a microbial community. Our approach using predominant culturable isolates from microaggregates combined with measurements of their soil aggregative ability appears to be a good starting point to provide information on microbial populations that can function as soil aggregators, since no molecular method is available to test for soil aggregation as this metabolic potential can have different genetic backgrounds. In addition, we acknowledge that the present study is on a single dryland soil and that further investigations on a variety of dryland soils are necessary. Also, more information on the influence of seasonal change on the abundance of specific microorganisms associated with aggregation is necessary to understand the variations in their communities under different tillage, crop rotation, and systems in semi-arid environments.

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